LONDA AND TRAUB LLP 20 Exchange Place, 37th Floor U8/983605 New York, N.Y. 10005

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21/Rec'd POTATIO 2,9 DEC 1997

Atty's Docket No. 2936.104/00

EXPRESS MAIL CERTIFICATION

58484302# "Express" Mail label number/

(A) Date of Deposit: December 29, 1997

I hereby certify that this transmittal letter and the papers and fees identified in this transmittal letter as being transmitted herewith are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated at (A) above and are addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231

Name of Person mailing the above: / Kathleen D. Monical

Signature of Person mailing the above item

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (D0/E0/US)

International Application No.:

PCT/DE96/01185

International Filing Date

27 June 1996 (27.06.96)

Priority Date Claimed

(28.06.95)28 June 1995

Title of Invention

Microsatellite Markers for Plants of the Species Triticum

Aestivum and Tribe Triticeae and

the Use of Said Markers

Applicant(s) for DO/EO/US

Marion Roder; Jens Plaschke;

and Martin Ganal

Applicant herewith submits to the United States Designed/Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ✓ This express request to immediately begin national examination procedures (35 U.S.C. 371(f).

2. 🗹 The U.S. National Fee (35 U.S.C. 371(c)(1) and other fees as follows:

TOTAL CLAIMS	- 20 =	CLAIMS OVER 20		RATE 22 =	TOTAL FEES FOR CLAIMS OVER 20
NUMBER OF INDEPENDENT CLAIMS 1	- 3 =	CLAIMS OVER 3		RATE 80 =	TOTAL FEES FOR INDEPENDENT CLAIMS OVER 3
MULTIPLE DEPENDENT CLAIM(S) PRESENT No			\$	RATE 260 per APPLN.	FEE MULTIPLE DEPENDENT CLAIM(S) \$
BASIC NATIONAL FEE International USPIO (37 CFR No Internation USPIO (37.CFR paid to USPIO Neither Intern (37 CFR 1.482) (37 CFR 1.485) International USPIO (37 CFR provisions of X Filing with an Surcharge of \$130 or oath or dec	preliminar 1.482) = al prelimi 1.482) but (37 CFR 1. ational pr nor Inter a)(2) paid preliminar 1.482) and PCT Articl EPO or JPO .00 for ful	y examinati \$ 700.00 nary Examin internatio 445 (a)(2) eliminary enational se to USPTO = y examinati all claims a)3(2)(2) b) search repositions the	ation nal se = \$ 7: xamin: arch 1 \$1040 on fee satiu to (4) port =	fee paid t earch fee 70.00 ation fee fee 0.00 e paid to sfied) = \$ 96.00 = \$ 930.00	
claimed priori	ty date (3	7 CFR 1.482	(e)).	au.iiest	
		TOTAL OF ABO		ALCULATIONS	\$ 930.00
Reduction by 1/2	for filing	by small e	ntity		<u> </u>
				SUBTOTAL	\$930.00
Process fee of \$13 translation la claimed priori	er than 20) mos. from	the e	nglish earliest	
		TOT	TAL NA	TIONAL FEE	\$ 930.00
Fee for recording	the enclos	ed assignme	nt		
		TOTA	I FEE	S ENCLOSED	\$ 930.00

a. ✓ A check in the amount of \$930.00 to cover the above fees is enclosed.
 b. Please charge my Deposit Account No. 04-2216 in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed.
 c. ✓ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 04-2216. A duplicate copy of this sheet is enclosed.

- A copy of the International Application as filed (35 U.S.C. 371(c)(2)

 a. __ is transmitted herewith (required only if not transmitted by the International Bureau).

 b. __ is not required, as the application was filed in the United States Receiving Office.

 c. __ has been transmitted by the International Bureau.
- x A translation of the International Application into English.
- Amendments to the claims of the International Application under PCT Article 19

 a. ___ are transmitted herewith (required only if not transmitted by the International Bureau).
 b. ___ have been transmitted by the International Bureau.
- __ A translation of the amendments to the claims under PCT Article 19 6.
- __ An oath or declaration of the inventor [35 U.S.C. 371(c)(4)] 7.
- $_$ A translation of the Annexes to the International Preliminary Examination Report under PCT Article 36 (35. U.S.C. 371(c)(5)).

Other document(s) or information included:

- X A Preliminary Amendment
 An assignment document for recording. Please mail the recorded assignment document to the undersigned.
- The above checked items are being transmitted
 a. __ before the 18th month publication.
 b. __ after publication and the Article 20 communication but before 20 months from the

 - after publication and the Article 20 communication but before 20 months from the priority date.
 after 20 months (surcharge and/or processing fee included).
 Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 20 months and no proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
 e. x
 by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
 f. after 30 months (surcharge and/or processing fee included).
 Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
- 12. At the time of transmittal, the time limit for amending claims under Article 19
 a. ___ has expired and no amendments were made.
 b. ___ has not yet expired.
- 13. ___Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on ___ namely:

Please direct all communications in connection with this application to the undersigned at LONDA AND TRAUB LLP

Exchange Place, 37th Floor New York, N.Y. 10005 20

Londa (33,531)

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PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No: 2936.104/00

Applicant(s) : Marion Roder

Filed : Concurrently herewith

For : Microsatellite Markers for Plants of the Species

Triticum Aestivum and Tribe Triticeae and the Use

of Said Markers

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents Washington, D.C. 20231

Dear Sir:

Prior to examination, please amend the application as

follows:

IN THE SPECIFICATION

Page 1, between lines 3 and 4, please insert

--Background of the Invention--;

Page 2, before line 1, please insert

--Summary of the Invention--;

Page 3, between lines 10 and 11, please insert

--Detailed Description of the Invention --.

IN THE CLAIMS

Claim 2, line 1, please delete "characterized in that" and
insert --wherein--;

Claim 3, line 1, please delete "characterized in that" and
insert --wherein--;

Claim 4, line 1, please delete "characterized in that" and
isert --wherein--;

Claim 5, line 1, please delete "characterized in that" and
insert --wherein--;

Claim 6 (amended) A method for the preparation of a microsatellite marker of [claims 1 to 5] <u>claim 1</u> for plants of the Triticum aestivum species as well of the Tribe Triticeae, [characterized in that] <u>wherein</u> hypervariable genome sections (so-called microsatellites), with the help of the polymerase chain reaction (PCR), are amplified, subsequently separated and detected to polymorphous fragments in the presence of two specific primers, which flank a microsatellite sequence to the left and right of each microsatellite locus.

Claim 7, line 1, please delete "characterized in that" and
insert --wherein--;

Claim 8, line 1, please delete "characterized in that" and insert --wherein--;

Please cancel claims 9 and 10.

REMARKS

The above amendments were made to place the application into proper United States patent format.

Early and favorable consideration of the application is respectfully requested.

Respectfully Submitted,

Bruce/S. Londa (33,531) Attorney for Applicant

Londa and Traub LLP

20 Exchange Place, 37th Floor

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klm0240



MAIL CERTIFICATION

PATENTS

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on February 13, 1998.

Bruce S. Londa

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No. 2936.104/00

EXAMINER

GROUP ART UNIT :

APPLICANT

: Marion Roder

APPLN. NUMBER

: 08/983,605

FILED

: December 29, 1997

FOR

: Microsatellite Markers for Plants of the Species Triticum Aestivum and

Tribe Aestivum and Tribe Triticeae and the Use of Said Markers

SUPPLEMENTARY PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

IN THE SPECIFICATION

<u>Page 13, line 7</u>, please delete "mappings" and insert --mapping--; and delete "distinguishing" and insert --trait analysis--;

\ line 3, delete "features";

line 10, please delete "this" and insert --these--; and delete "marker" and insert --markers--;

Page 14, line 3, please delete "minute" and insert --minutes--;

Page 15, line 24, please delete "minutes" and insert --minutes--.

REMARKS

The above amendments were made to correct grammatical and translation errors. Early and favorable consideration of this application is earnestly solicited.

Respectfully Submitted,

Bruce S. Londa (33,531) Attorney for Applicant Londa and Traub LLP

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08/983605

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PCT/DE96/01185

2936.104/00

TRITICUM AESTIVUM AND TRIBE TRITICEAE AND THE USE OF SAID MARKERS

The invention relates to novel genetic markers for wheats (Triticum aestivum L.) and closely related species (Tribus Triticeae) and to the use of said markers.

The most widely spread, known, DNA-based genetic markers are the so-called restriction fragment length polymorphisms (RFLP) markers. For using these markers, genomic DNA is digested with restriction enzymes, separated on agarose gels and transferred to nylon membranes (Southern Blot). Specific fragments are detected by hybridization with radioactively labeled DNA probes. When mutations occur in the region of the restriction enzymes used or when smaller deletions/insertions occur, polymorphisms between different lines are found, which are passed on stably and mostly codominantly. The use of RFLP markers in hexaploid cultivated wheat is possible only to a limited extent, since only very little polymorphism is detected in wheat in this manner.

It has already been described that microsatellite markers detect significantly more polymorphism between different wheat lines than do RFLP markers. This can be attributed particularly to the occurrence of multiple alleles per locus (Röder et al., Mol. Gen. Genet. (1995) 246, 327 - 333). Moreover, it is known that microsatellite markers have the advantage that they can be detected by way of PCR and that therefore large amounts of samples can be analyzed more easily.

It is an object of the invention to provide novel microsatellite markers for the genetic analysis of plants of the Triticum aestivum species, which markers are distinguished by a degree of DNA polymorphism, which is higher than that of other molecular probes, that have been developed previously for the wheat genome.

This objective is accomplished by claims 1 to 10. The inventive markers are based on the amplification of certain hypervariable genome sections, the so-called microsatellites, with the help of their polymerase chain reaction (PCR). For specific amplification, two primers, in each to the case left and the right in the flanking sequences, are required for each microsatellite locus. On the average, these primers are 20 ± 3 bases long and are defined by their sequences. In principle, a microsatellite marker is a sequence tagged site (STS), which is defined by two specific primers. These primers flank, in each case to the left and the right, a socalled microsatellite sequence. A microsatellite sequence is defined as a tandem repetitive repetition of a di-, tri- or tetranucleotide sequence, for example (GA)_n, in which $n \ge 10$. Composite microsatellite sequences also occur, such as $(GT)_n(AT)_n$, as well as imperfect sequences, in which individual bases are mutated, such as (GA)_nCA(GA)_n. Among various lines and varieties, there is variation in the number of repeats at a certain locus. After amplification of the microsatellites, this leads, by means of the specific primers in the flanking sequences, to PCR products of different length and, with that, to polymorphisms. These polymorphisms are passed on stably and can therefore be used as genetic markers. In some cases, null alleles (no visible fragment) also occur, when there are mutations within the binding site for the primers.

The separation and detection of the PCR products obtained can be carried out with different technical variants. For separating the fragments, highly resolving agarose gels, native polyacrylamide gels or denaturing polyacrylamide gels (= sequencing gels) can be used. Depending on the separation system, fragments are detected using ethidium bromide staining, silver staining or, after labeling the PCR

fragments radioactively, using autoradiography. A further, very effective variation for separation and detection consists of the use of an automatic sequencer with dye- or fluorescence-labeled primers. For this purpose, it is necessary to synthesize a dye- or fluorescence-labeled primer from each microsatellite primer pair. PCR amplification results in a labeled product, which can be detected by the sequencing equipment. At the same time, dye- or fluorescence-labeled size standards are also separated for each sample in the same track. After that, special software enable the absolute size of each fragment, which has been separated, to be calculated and, with that, also permits fragments from different gel runs to be compared. With this method, several hundred samples can be analyzed largely automatically in a day.

Pursuant to the invention, microsatellite markers are made available, which contain the following primer pairs with assigned microsatellite sequences or a number thereof and amplify the loci of all chromosomes of the wheat genome and therefore find use for gene marking.

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Substitute Page (Rule 26)

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WMS550	5' CCC ACA AGA ACC TTT GAA GA 3'	5' CAT TGT GTG TGC AAG GCA C 3'	150	C1, A1C1, C1) (f
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WMS565	5' GCG TCA GAT ATG CCT ACC TAG G 3'	5' AGT GAG TTA GCC CTG AGC CA 2	201	dillini, 12	ر د د
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WMS569	5' GGA AAC TTA TTG ATT GAA AT 3'	5' TCA ATT TTG ACA GAA GAA TT 3'	134		ر ان در
WMS570	5' TCG CCT ITT ACA GTC GGC 3'	5' ATG GGT AGC TGA GAG CCA AA 3'	143	ن	ر د د د
WMS573	5' AAG AGA TAA CAT GCA AGA AA 3'	5' TTC AAA TAT GTG GGA ACT AC 3'	217		رم رد در در
WMS577	5' ATG GCA TAA TTT GGT GAA ATT G 3'	5' TGT TTC AAG CCC AAC TTC TAT T 3'	133		ع الراد دوري
WMS582	5' AAG CAC TAC GAA AAT ATG AC 3'	5' TCT TAA GGG GTG TTA TCA TA 3'	151	CA,1A	رد چوروم
WMS583	5' TTC ACA CCC AAC CAA TAG CA 3'	5' TCT AGG CAG ACA CAT GCC TG 3'	591		ران دوره
WMS588	5' GAT CCC CAA TTG CAT GTT G 3'	5' CTT GCA ACT GGG GGA CAC 3'	102		00 ر و0 °ر
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'CS' Weizensorte 'Chinese Spring'

These markers are distinguished by a high degree of polymorphism between different wheat varieties or lines and usually detect several alleles per genetic locus in different wheat lines.

They can therefore be used for DNA fingerprinting, species identification, relationship or similarity studies, characterization of cytological lines, such as deletion lines, substitution lines, addition lines, etc. and all forms of genetic mappings, including mapping of individual genes and quantitative distinguishing features (QTLs). In addition, their use is also very suitable for automation and it is possible to carry out the detection of the products with nonradioactive methods.

With the help of this inventive marker, the possibility is provided, for example, of differentiating almost all European wheat lines.

The invention is described in greater detail below by means of examples.

1. Amplification of the Microsatellite Markers

The microsatellite markers are amplified according to the following protocol:

10 mM tris-HCl, pH 8

50 mM KCl

1.5 mM MgCl₂ (in a few exceptional cases 3 mM MgCl₂)

0.01% (w/v) gelatin

0.2 mM of each desoxynucleotide

250 nM of each primer (in each case to the left and right of a pair)

1 - 2 units taq polymerase

50 - 150 ng matrixes (template) DNA

are amplified in a volume of 25 or 50 μ L according to the following profile:

92°C	3 minute	
92°C	1 minute (denaturing phase)	
60°C	1 minutes (annealing phase)	45 cycles
72°C	2 minutes (elongation phase)	

72°C 10 minutes (extension phase)

The amplification takes place in a Perkin Elmer 9600 with lid heating or in an MJ Research Thermocycler without lid heating. In this apparatus, a layer of mineral oil is placed over the reactions. The temperature of the annealing phase depends on the melting point (T_m) of the primer and in some cases even is 50°C or 55°C.

2. Separation of the Microsatellite Markers on Polyacrylamide Gels, Which Are Not Denaturing

The PCR reactions are mixed with 1/10 volume of stop buffer (0.02 M tris acetate of pH 8.1, 0.025 M sodium acetate, 0.02 M EDTA, 70% glycerin, 0.2% SDS, 0.6% bromphenol blue, 0.6% xylene cyanol) and in each case 25 μ L are separated in 10% polyacrylamide gels (1.5 mm thick, 18 cm long).

Formulation for polyacrylamide gel (10%):

25 mL stock acrylamide solution (19 g acrylamide, 1 g bisacrylamide, diluted to 100 mL with water)

10 mL 5X TBE (1X TBE = 0.09 M tris borate of pH 8.3, 0.002 M EDTA 15 mL water

are mixed and the polymerization is started by the addition of 220 μ L of ammonium persulfate (10%, freshly prepared) and 20 μ L of TEMED. Immediately after the addition, the mixture is poured into the sealed gel mold and the comb for forming pockets is inserted. The polymerization is completed after about 1 hour. The gel is placed in the gel chamber and a preliminary run is carried out without samples for about 30 minutes at 150 volts in 1X TBE. After that, the samples are loaded (25 μ L of each) and the separation is carried out for 14 - 16 hours at 100 volts.

After the electrophoresis is completed, the gel is stained for about minutes in ethidium bromide (1 - 2 drops of 10 mg/mL in 1 liter of water) and the fragments are made visible by a UV transilluminator and documented.

3. Separation of Microsatellite Markers on Denaturing Gels

For the separation of the amplified fragments on denaturing gels, an automatic laser fluorescence (A.L.F.) sequencer (Pharmacia), for example, is used. In order to enable the fragments to be detected by means of a laser, one primer per pair is marked at the 5' end with fluorescein. Per PCR reaction, 0.3 to 1.5 microliters are mixed with 2.5 microliters of stop buffer (deionized formamide; 5 mg/mL dextran blue), denatured (1 minute; 90°C) and loaded onto the gel. Gel plates with a 9 cm separation distance are used, as recommended by the manufacturer for the fragment analysis. The gel solution contains 6.5% Long-Ranger (AT Biochem), 7M urea and 1.2X TBE buffer. The gels are 0.35 or 0.5 mm thick. The conditions for the gel run are 600 V, 40 mA, 50 W, 0.84 s data collection interval and 2 mW laser energy. The gel runs are ended after about 80 to 90 minuutes. This is sufficient for detecting fragments up to a size of 300 bp. A gel can be used for four or five runs. For each gel

run, a data set is obtained. With this data set and by means of internal size standards, the exact fragment sizes are determined in the computer program Fragment Manager (Pharmacia) and thus the smallest size differences of a base pair are determined.

Claims

- 1. Microsatellite markers (based on hypervariable genome sections) for plants of the Triticum aestivum species, as well as of the Tribe Triticeae using the polymerase chain reaction (PCR), characterized in that a sequence tagged site (STS), which is defined by two specific primers, which average a length of 20 ± 3 bases and flank a microsatellite sequence, which microsatellite markers are amplified to polymorphisms (PCR products of different length).
- 2. The microsatellite markers of claim 1, characterized in that the microsatellite sequence is a tandem-repetitive n-fold repetition of a di-, tri- or tetranucleotide sequence, in which $n \ge 10$.
- 3. The microsatellite markers of claim 1, characterized in that the microsatellite sequence is a composite microsatellite sequence.
- 4. The microsatellite markers of claim 1, characterized in that the microsatellite sequence is an imperfect sequence, in which individual bases are mutated.
- 5. The microsatellite markers of claim 1, characterized in that the following primer pairs with assigned microsatellite sequences or a number thereof are contained.

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	WMS Number	WMS Primer left WMS Primer links	WMS Primer Right WMS Primer rechts	Repeat Type Repcat-Typ
	WMS052	5' CTA TGA GGC GGA GGT TGA AG 3'	5' TGC GGT GCT CTT CCA TTT 3'	GTimp
	WMS055	5' GCA TCT GGT ACA CTA GCT GCC 3'	S' TCA TGG ATG CAT CAC ATC CT 3	CTimp
	WMS057	5' TCG ATT CTG AAA GGT TCA TCG 3'	5' CGA TCA AGT AGT TGA AAG CGC 3'	AAAAAimp
	WMS058	5' TCT GAT CCC GTG AGT GTA ACA 3'	5' GAA AAA AAT TGC ATA TGA GCC C 3'	CA
	WMS060	S' TGT CCT ACA CGG ACC ACG T 3'	5' GCA TTG ACA GAT GCA CAC G 3'	CA
	WMS063	S' TCG ACC TGA TCG CCC CTA 3'	5' CGC CCT GGG TGA TGA ATA GT 3'	GAA,CA,TA
	WMS067	5' ACC ACA CAA ACA AGG TAA GCG 3'	S' CAA CCC TCT TAA TTT TGT TGG G 3'	CA
	WMS068	5' AGG CCA GAA TCT GGG AAT G 3'	5' CTC CCT AGA TGG GAG AAG GG 3'	GA
	WMS070	5' AGT GGC TGG GAG AGT GTC AT 3'	5' GCC CAT TAC CGA GGA CAC 3'	CT
_	WMS071	5' GGC AGA GCA GCG AGA CTC 3'	5' CAA GTG GAG CAT TAG GTA CAC G 3'	GT
,	WMS077	5' ACA AAG GTA AGC AGC ACC TG 3'	5' ACC CTC TTG CCC GTG TTG 3'	CA,GA
	WMS082	S' ACG TTA GAA GGT GCA ATG GG 3'	5' AGT GGA TGC ACC GAC TITI G 3'	GT,GAimp
	WMS088	S' CAC TAC AAC TAT GCG CTC GC 3'	S' TCC ATT GGC TTC TCT CTC AA 3'	GT
	WMS095	5' GAT CAA ACA CAC ACC CCT CC 3'	S' AAT GCA AAG TGA AAA ACC CG 3'	CA
_	WMS099	5' AAG ATG GAC GTA TGC ATC ACA 3'	S' GCC ATA TITI GAT GAC GCA TA 3'	CA
	WMS102	S' TCT CCC ATC CAA CGC CTC 3'	S'TGT TGG TGG CTT GAC TAT TG 3'	CT
,_	WMS106	S' CTG TTC TTG CGT GGC ATT AA 3'	S' AAT AAG GAC ACA ATT GGG ATG G3'	GA
	WMS107	5' ATT AAT ACC TGA GGG AGG TGC 3'	S' GGT CTC AGG AGC AAG AAC AC 3'	CI
	WMS108	5' CGA CAA TGG GGT CTT AGC AT 3'	5' TGC ACA CTT AAA TTA CAT CCG C 3'	GTimp
o c \	WMS111	5' TCT GTA GGC TCT CTC CGA CTG 3'	S' ACC TGA TCA GAT CCC ACT CG 3'	cr, Gr
	WMS112	5' CTA AAC ACG ACA GCG GTG G 3'	S' GAT ATG TGA GCA GCG GTC AG 3'	CTimp
	WMS113	S' ATT CGA GGT TAG GAG GAA GAG G3'	S' GAG GGT CGG CCT ATA AGA CC 3'	-CT
÷,	WMS114	S' ACA AAC AGA AAA TCA AAA CCC G 3'	S' ATC CAT CGC CAT TGG AGT G 3'	GA
	WMS118	S' GAT GTT GCC ACT TGA GCA TG 3'	5' GAT TAG TCA AAT GGA ACA CCC C 3'	CA
	WMS119	S' TGA CTA ACA TCC TTT GTC ACG C 3'	S' CAT GTC TCA ACC ACC CAC AG 3'	GTimp
	WMS120	S' GAT CCA CCT TCC TCT CTC TC 3'	5' GAT TAT ACT GGT GCC GAA AC 3'	CT,CA
-	. WMS121	S' TCC TCT ACA AAC AAA CAC AC 3'	S' CTC GCA ACT AGA GGT GTA TG 3'	CA

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	WMS122	5' GGG TGG GAG AAA GGA GAT G 3'	S' AAA CCA TCC TCC ATC CTG G 3'	CT,CA
	WMS124	5' GCC ATG GCT ATC ACC CAG 3'	5' ACT GTT CGG TGC AAT TTG AG 3'	CT,GTimp
	WMS126	S' CAC ACG CTC CAC CAT GAC 3'	S' GTT GAG TTG ATG CGG GAG G 3'	CA
	WMS128	5' AGC ACA TTT TAA CAC AGA TA 3'	5' ATC TGT GAA ATT TTG AAA AC 3'	CA
	WMS129	5' TCA GTG GGC AAG CTA CAC AG 3'	5' AAA ACT TAG TAG CCG CGT 3'	GTimp
	WMS130	5' AGC TCT GCT TCA CGA GGA AG 3'	5' CTC CTC TTT ATA TCG CGT CCC 3'	CT
	WMS131	S' AAT CCC CAC CGA TTC TTC TC 3'	S' AGT TCG TGG GTC TCT GAT GG 3'	CT
	WMS132	S' TAC CAA ATC GAA ACA CAT CAG G 3'	S' CAT ATC AAG GTC TCC TTC CCC 3'	GA,GAA
	WMS133	S' ATC TAA ACA AGA CGG CGG TG 3'	5' ATC TGT GAC AAC CGG TGA GA 3'	CT
S	WMS134	S' CAT GGA ACT TAG ACA GAA TTG 3'	S' CAG TAC TTG GTA CTG AAC AGG 3'	CA
ub E F	WMS135	S' TGT CAA CAT CGT TTT GAA AAG G 3'	5' ACA CTG TCA ACC TGG CAA TG 3'	СA
st: RSA	WMS136	S' GAC AGC ACC TTG CCC TTT G 3'	S' CAT CGG CAA CAT GCT CAT C 3'	CT
itu TZB	WMS140	5' ATG GAG ATA TTT GGC CTA CAA C 3'	S' CTT GAC TTC AAG GCG TGA CA 3'	CI
ite LA7	WMS144	5' TTT GCT GTG GTA CGA AAC ATA C 3'	S' ACT CAC AAA TGT CTA ATA AAA C 3'	GT
· P.	WMS146	S' CCA AAA AAA CTG CCT GCA TG 3'	S' CTC TGG CAT TGC TCC TTG G 3'	GAimp
age REG	WMS148	5' GTG AGG CAG CAA GAG AGA AA 3'	5' CAA AGC TTG ACT CAG ACC AAA 3'	CA
	WMS149	S' CAT TGT TTT CTG CCT CTA GCC 3'	5' CTA GCA TCG AAC CTG AAC AAG 3'	GA
	WMS153	5' GAT CTC GTC ACC CGG AAT TC 3'	5' TGG TAG AGA AGG ACG GAG AG 3'	GA
le	WMS154	5' TCA CAG AGA GAG AGG GAG GG 3'	5' ATG TGT ACA TGT TGC CTG CA 3'	GA
26	WMS155	5' CAA TCA TTT CCC CCT CCC 3'	S' AAT CAT TGG AAA TCC ATA TGC C 3'	CŢ
5)	WMS156	5' CCA ACC GTG CTA TTA GTC ATT C 3'	S' CAA TGC AGG CCC TCC TAA C 3'	CT
	WMS157	S' GTC GCG GTA AGC TTG 3'	5' GAG TGA ACA CAC GAG GCT TG 3'	CT
	WMS159	S' GGG CCA ACA CTG GAA CAC 3'	5' GCA GAA GCT TGT TGG TAG GC 3'	CT
÷;	WMS160	5' TTC AAT TCA GTC TTG GCT TGG 3'	S' CTG CAG GAA AAA AAG TAC ACC C 3'	QA
	. WMS161	5' GAT CGA GTG ATG GCA GAT GG 3'	S' TGT GAA TTA CTT GGA CGT GG 3'	CT
	WMS162	5' AGT GGA TCG ACA AGG CTC TG 3'	5' AGA AGA AGC AAA GCC TTC CC 3'	CA
	WMS163	S' ACC TCG ACA GAC CTG GTA CG 3'	5' GTC TTT GTC ACC CGA TGG AC 3'	CI
	WMS164	S' ACA TTT CTC CCC CAT CGT C 3'	S' TTG TAA ACA AAT CGC ATG CG 3'	C

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WMS165	5' TGC AGT GGT CAG ATG TIT CC 3'	SCITTICITICAGAII OCO CC 3	K
WMS169	5' ACC ACT GCA GAG AAC ACA TAC G 3'	5' GTG CTC TGC TCT AAG TGT GGG 3'	СA
WMS174	S' GGG TTC CTA TCT GGT AAA TCC C 3'	5' GAC ACA CAT GTT CCT GCC AC 3'	CT
WMS179	5' AAG TTG AGT TGA TGC GGG AG 3'	5' CCA TGA CCA GCA TCC AC '' C' 3'	GT
WMS180	5' ATC CGC CTA AGG AAT AGT GT 3'	5' GAT CGC ACG GGA GAG AG,	CT
WMS181	5' TCA TTG GTA ATG AGG AGA GA 3'	S' GAA CCA TTC ATG TGC ATG TC 3'	В
WMS182	5' TGA TGT AGT GAG CCC ATA GGC 3'	5' TTG CAC ACA GCC AAA TAA GG 3'	CT
WMS186	5' GCA GAG CCT GGT TCA AAA AG 3'	S' CGC CTC TAG CGA GAG CTA TG S'	СA
WMS189	5' AGG AGC AGC GGA ACG AAC 3'	5' AGA AAT ACG GAA ACC CAC CC 3'	CA
WMS190	5' GTG CTT GCT GAG CTA TGA GTC 3'	5' GTG CCA CGT GGT ACC TTT G 3'	CT,GT
WMS191	5' AGA CTG TTG TTT GCG GGC 3'	S' TAG CAC GAC AGT TGT ATG CAT G 3'	CT
WMS192	5' GGT TTT CTT TCA GAT TGC GC 3'	5' CGT TGT CTA ATC TTG CCT TGC 3'	CT
WMS193	S' CTT TGT GCA CCT CTC TCT CC 3'	S' AAT TGT GTT GAT GAT TTG GGG 3'	CT,CA
WMS194	5' GAT CTG CTC TAC TCT CCT CC 3'	5' CGA CGC AGA ACT TAA ACA AG 3'	CT
WMS195	5' AGG TGC CGT CGC GTC TAC 3'	5' ACC CCC CAC GTC AGA GAG 3'	CT
WMS197	S' GAG AAA GAG GTC TGG AGG TCG 3'	S' CAA AAT GCA CAA GAA TGG AGG 3'	CT
WMS198	S' TTG AAC CGG AAG GAG TAC AG 3'	S' TCA GTT TAT TTT GGG CAT GTG 3'	CA
WMS200	5' TCA ACG GAA CAG ATG AGC G 3'	S' GAC CTG-ATG AGA GCA AGC AC 3'	CT
WMS203	5' CCC AAA GCA GCG CAA GC 3'	S' ACC AAT GCT ATC GGC TCG 3'	CA,GA
WMS205	5' CGA CCC GGT TCA CTT CAG 3'	S' AGT CGC CGT TGT ATA GTG CC 3'	CT
WMS210	5' TGC ATC AAG AAT AGT GTG GAA G 3'	S' TGA GAG GAA GGC TCA CAC CT 3'	СА
WMS212	5' AAG CAA CAT TTG CTG CAA TG 3'	S' TGC AGT TAA CTT GTT GAA AGG A 3'	CT
WMS213	S' TGC CTG GCT CGT TCT ATC TC 3'	S' CTA GCT TAG CAC TGT CGC CC 3'	ВA
WMS218	5' CGG CAA ACG GAT ATC GAC 3'	S' AAC AGT AAC TCT CGC CAT AGC C 3'	CT
, WMS219	5' GAT GAG CGA CAC CTA GCC TC 3'	S' GGG GTC CGA GTC CAC AAC 3'	GAimp
WMS224	5' TGA GTC CAG CAC TGC TGC 3'	S' CAA CAT CCG CTC GTA TTC AA 3'	CT
WMS228	5' TCA TAT GCA CCT CTT TCC TAG G 3'	S' GTG TGC CAC CTT TGA CGT C 3'	CT,CA
. WMS231	5' AGC TCG GGA TGA AGC GTG 3'	5' GAT CCG CCG CTG CGT TT 3'	GAimp

5' TGC TCT TTG GCG AAT ATA TGG 3'

5' CAA GAT CGT GGA GCC AGC 3'

WMS269

WMS271 WMS272 WMS273 WMS274

5' ATT GGA CGG ACA GAT GCT TT 3'

AAC TTG CAA AAC TGT TCT GA 3'

5' GAG AAA CAT GCC GAA CAA CA 3'

S' CTC CCT GTA CGC CTA AGG C 3'

WMS261 WMS263 WMS264

WMS260

S' GCC CCC TTG CAC AAA TC 3'

S' TCT GCC GTA AGT CGC CTC 3'

5' TGT TGC GGA TGG TCA CTA TT 3'

WMS265 WMS268

S'TCA ACC GTG TGT AAT TTT GTC C 3'

5' TCA AAA CAT AAA TGT TCA TTG GA 3'

5' ATC TCA ACG GCA AGC CG 3'

WMS232 WMS233

S' CTG ATG CAA GCA ATC CAC C 3'

5' CTC ATT GGG GTG TGT ACG TG 3'

CAimp GAimp GA S'TTT GAG CTC CAA AGT GAG TTA GC 3' 5' GTT CAA AAC AAA TTA AAA GGC CC3' 5' GCA TGC ATG AGA ATA GGA ACT G 3' 5' TTA TGT GAT TGC GTA CGT ACC C 3' S' CTT CCA TGG ACT ACA TAC TAG C 3' S' CTG CCA TTT TTC TGG ATC TAC C 3' S' GAG TAC ACA TTT GGC CTC TGC 3' S' GGG ATG TCT GTT CCA TCT TAG 3' S' AGC TGC TAG CTT TTG GGA CA 3' 5' CCA AGA CGA TGC TGA AGT CA 3' S' AGC AGT GAG GAA GGG GAT C 3' 5' TAT TTG AAG CGG TITI GAT TT 3' S' GGT TTC ATT GCT TGC CCT AA 3' S' CTG GAT GCA TCA CAT CCA AC 3' 5' TGT TGT TGG CCT GTA TGC AT 3' S' TGG CGT GGT CTA AAT GGA C 3' S' CTC GCG CTA CTA GCC ATT G 3' 5' TCT GCC GTA AGT CGC CTC 3' S' TTT GGA CAT TTC CCA GCG 3' 5' ATC TGT CCA TTC GAG CGC 3' 5' GTA CAC GCC GTA GGC CC 3' 5' CGA CCG ACT TCG GGT TC 3' S' CGC AGC TAC AGG AGG CC3' S' ATG TGC ATG TCG GAC GC 3' 5' AGT GCC TTG CCG AGG TC 3' S' TGC ATA TAA ACA GTC ACA CAC CC 3' 5' CAA CTG TAC GTA GGT TTC ATT GC 3' 5' AGG GAA AAG ACA TCT TTT TC 3' 5' GAA TCA CTT GTG AAG CAT CTG G 3' S' GAG TCC TGA TGT GAA GCT GTT G 3' S' GAT CGC TTC ATC TCT CTC TCT C 3' 5' AGG GGA TAT GTT GTC ACT CCA 3'

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Substitute Page (Rule 26)

WMS259

WMS255

VMS257 WMS258

5' CAA CTG GTT GCT ACA CAA GCA 3'

S' AGA GTG CAT GGT GGG ACG 3'

S' CAA ATG GAT CGA GAA AGG GA 3'

5' AGG ACT TCC GCA CCC TG 3'

VMS248 VMS249 WMS251

S' GCA ATC TITI TITI CTG ACC ACG 3'

S' CAG CGC AGT TAG CTC GC 3'

WMS245

WMS247

WMS244

S' TCT TCC AAC TAA AGC ATA GC 3'

S' TCG CTT CTA CCG CTC ACC 3'

WMS237

WMS234

WMS238

5' TCC AAG GCA GTA GGC AGG 3' 5' GGC AGC TGA GGC AAT CTG 3'

WMS242

WMS241

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WAG 275	5' AAT TIT CIT CCT CAC ITA ITC I 3'	5' AAC AAA AAA TTA GGG CC 3'	CT
27.28MM	4' ATT TGC CTG AAG AAA ATA TT 3'	5' AAT TTC ACT GCA TAC ACA AG 3'	CT
0/25/WW	STORT COLL TO A TIGA ACCIONA 31	5' CTG CCC AAT TTT CTC CAC TC 3'	GTimpGAimp
WIVIS218	S COO COA TAT TTC TGT AAG TAT GC 3'	5' GCA GGT AAT GGC CGG AC 3'	GT
WM5281	S COO CCA IAI 11C 1CI III C II	5' TCT CAT TCA CAC ACA ACA CTA GC 3'	GA
787SWM	STINGUE CITATION SECTION STATES STATES AND ACCOUNT OF SECTION	5' GCA CAT TIT TCA CIT TCG GG 3'	GA
WW2284	S' ATG ACC CTT CTG CCA AAC AC 3'	5' ATC GAC CGG GAT CTA GCC 3'	GA
CHISTON W	S' CAT CCC TAC GCC ACT CTG C 3'	5' AAT GGT ATC TAT TCC GAC CCG 3'	CA
WMS299	5' TCA CCG TGG TCA CCG AC 3'	S' CCA CCG AGC CGA TAA TGT AC 3'	CT
WMS293	5' TAC TGG TTC ACA TTG GTG CG 3'	5' TCG CCA TCA CTC GTT CAA G 3'	CA
WMS294	5' GGA TTG GAG TTA AGA GAG AAC CG 3'	5' GCA GAG TGA TCA ATG CCA GA 3'	GAimp
WMS295	5' GTG AAG CAG ACC CAC AAC AC 3'	5' GAC GGC TGC GAC GTA GAG 3'	ВA
WMS296	5' AAT TCA ACC TAC CAA TCT CTG 3'	5' GCC TAA TAA ACT GAA AAC GAG 3'	CT
WMS297	S' ATC GTC ACG TAT TTT GCA ATG 3'	5' TGC GTA AGT CTA GCA TTT TCT G 3'	GT, GA
WAG299	5' ACT ACT TAG GCC TCC CGC C 3'	5' TGA CCC ACT TGC AAT TCA TC 3'	GA, TAG
WMS301	S' GAG GAG TAA GAC ACA TGC CC 3'	5' GTG GCT GGA GAT TCA GGT TC 3'	GA,G
WMS302	S' GCA AGC AAC AGC AGT AAC 3'	5' CAG ATG CTC TTC TCT GCT GG 3'	QΛ
WINISOL	S' AGG AAA CAG AAA TAT CGC GG 3'	5' AGG ACT GTG GGG AAT GAA TG 3'	CT
WMS311	STACE THE GOA AGA CGC TCC 3'	5' CTA CGT GCA CCA CCA TTT TG 3'	СА
TI COPYIN	STATE GEAT THE ACT TAG ACT	S' ACA TGC ATG CCT ACC TAA TGG 3'	СА
WM5312	S' CCG CCC TCA TTA AGT TTC AC 3'	5' TTT GAC AAG TAC ACG AGT CTG C 3'	CT,GT
WASSL	S AGG AGC TCC TCT GTG CCA C 3'	S' TTC GGG ACT CTC TTC CCT G 3'	CT
WINIS314	S CAT GGA CAT TITT ACC ACA AGA C 3'	S' TGC GTG TGG TCC ACC TC 3'	AT,GT
01631W	si GGT TGC TGT ACA AGT GTT CAC G 3'	5' CGG GTG CTG TGT GTA ATG AC 3'	Ct
WW.5330	4 CGA GAT ACT ATG GAA GGT GAG G 3'	5' ATC TTT GCA AGG ATT GCC C 3'	GT,GA
WIM5320	S'CAA TGT GGA GAC GGT GTG C 3'	S' TGT TGC ATG CGA TCA TGC 3'	GT,GAimp
WMS322	S'TCA CAA AAT GAT TTC TCA TCC G 3'	5' TGC AGA AAA CCA ACA AGG G 3'	СА
WMS325	S' TITI CITI CIG TCG TTC TCT TCC C 3"	5' TITI TTA CGC GTC AAC GAC G 3'	CT

Substitute Page (Rule 26)

11/1/01/18	5' GCA ATC CAC GAG AAG AGA GG 3'	5' CAC AAA CTC TTG ACA TGT GCG 3'	GT
WINIS226	stand CTA TCC ATG TGC CAG AG 3'	5' ACA TGT TTC ATG CAG GTA GCC 3'	GTT
WMS330	STIGGIA ICCATOLOGO CONTRA	5' AGT GCT GGA AAG AGT AGT GAA GC 3'	GA
WMS332	S' AUC CAU CAA UI C'ACC AMATAC.	S' TITL CAG TITL GCG TTA AGC TITL G 3'	GA
WMS333	St GCC CGG TCA TGT AAA ACG 3	SIAN ATC TCT TTT TAG CTA TC 3'	GA
WMS334	5' AAT TTC AAA AAG GAG AGA GA 3'	S' AAC AIG IGI III ING CITTURE	GA.GCGT
WMS335	5' CGT ACT CCA CTC CAC ACG G 3'	S'CGG ICC AAG IGC IAC CIT I C.	£ 15.
WMS336	S' CCC TIT AAT CTC GCT CCC TC 3'	SIGTOTOTIC TO USE THE CAN US	1) L) V L)
WMS337	5' CCT CTT CCT CCC TCA CTT AGC 3'	5' TGC TAA CTG GCC TTT GCC 3'	CI,CACI,CA
9113WW	S' AAT TIT CIT CCT CAC 1TA TT 3'	5' AAA CGA ACA ACC ACT CAA TC 3'	ct
WMS330	S' GCA ATC TTT TTT CTG ACC ACG 3'	5' ACG AGG CAA GAA CAC ACA TG 3'	СА
WAGATI	S' TTC AGT GGT AGC GGT CGA G 3'	5' CCG ACA TCT CAT GGA TCC AC 3'	CT
TECCINIAN (ATATICA GAGICAG ACGIGACIG3'	5' GGT CTA GCT TCG ACG ACA CC 3'	CT
WMS342	TAT CCA CITC CITC CITC CITC CITC CITC CI	5' ATT TGA GTC TGA AGT TTG CA 3'	GT
WMS.344	S CAN GOA AGG THT CGT TITL ATC C 3'	5' GCA TGT GGT CCA TGT ACT GC 3'	AT,GT
WMS346	S CAN UCA AUG 111 COL 111 CO. S.	S' ATC GGT GCG TAC CAT CCT AC 3'	GA
WMS349	S. COC TIC CAU ANA ACA ACA GOS	SI GCA TGG ATA GGA CGC CC 3'	GT
, WMS350	S' ACC TCA TCC ACA TGT TCT ACG 3	COLUMN COLUMN ACTIVITY OF A POLICY ACTIVITY ACTIVITY OF A POLICY ACTIVITY ACTIVITY AND A POLICY ACTIVITY ACTIVITY AND A POLICY ACTIVITY AND A PO	GCGT.GT
WMS353	S' CCA TGT TGA GTA GGT TCA GCC 3'	STOLI GOULTAG AND CITA CONTROL	C. A. C.
WMS356	5' AGC GTT CTT GGG AAT TAG AGA 3'	S' CCA ATC AGC CTG CAA CAA C.S.	v o
WASST	5' TAT GGT CAA AGT TGG ACC TCG 3'	5' AGG CTG CAG CTC TTC TFC AG 3'	GA
WAGSER	S' AAA CAG CGG ATT TCA TCG AG 3'	5' TCC GCT GTT GTT CTG ATC TC 3'	GAimp
0555MW	SI CTA ATT GCA ACA GGT CAT GGG 31	5' TAC TTG TGT TCT GGG ACA ATG G 3'	CT,CTTimp
WW3339	S CIA ACT TGT TGC CAA AGG GG 3'	5' ACA AAG TGG CAA AAG GAG ACA 3'	GAimp
10551MW	S OLY ACT TOL TOL CONTROL OF STATE ATT OF ST	5' AAT AAA ACC ATG AGC TCA CTT GC 3'	AT
WMS368	S CCA 111 CAC CIA AIG CC1 CC2	st ACC GTG GGT GTT GTG AGC 3'	CTimp
WMS369	S' CTG CAG GCC ATG ATG ATG S	S ACC GIG GOT TOO THE GIA CC 3'	CA, GA
WMS371	5' GAC CAA GAT ATT CAA ACT GGC C 3'	S AUC ICA COL TOC TIC CITIC STORY	QA
WMS372	S' AAT AGA GCC CTG GGA CTG GG 3'	S GAA GGA COA CAL TOC ACC TOC	L
WMS374	5' ATA GTG TGT TGC ATG CTG TGT G 3'	STECT AAT TAG COLLING CLOS	5 3
WMS375	5' ATT GGC GAC TCT AGC ATA TAC G 3'	5' GGG ATG TCT GTT CCA TCT TAG C. 3'	CA

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CT,CA,CA GAimp CA,GA CT,GT CT,GT GT £ g IJ IJ CA 5' CCT TAA CAC TTG CTG GTA GTG A 3' 5' ACG AAA TAC ACA AGT GGG ACA 3' S' GTA TAA TTC GTT CAC AGC ACG C 3' 5' TCA TGT CAA CTC AAG AAC ACG 3' S' CCA TGA TTT ATA AAT TCC ACC 3' 5' GCC AAG TTT CTT AGC TAG TTA A 3' 5' GAT GTC CAA CAG TTA GCT TA 3' 5' TTT AAG GAC CTA CAT GAC AC 3' 5' CTG CAC TCT CGG TAT ACC AGC 3' 5' ATA AAA CAG TGC GGT CCA GG 3' 5' CGA GAC CTT GAG GGT CTA GA 3' 5' GAC ATC AAT AAC CGT GGA TGG 3' 5' CGA CAG TCG TCA CTT GCC TA 3' 5' AGT GTG TTC ATT TGA CAG TT 3' 5' CAC CGC GTC AAC TAC TTA AGC 3' 5' TGA CAA GTA CAC GAG TCT GC 3' 5' TAC CAA CAC CCT AGC CCT TG 3' 5' TGC CAT GCA CAT TAG CAG AT 3' 5' TGT AGG CAC TGC TTG GGA G 3' 5' GAT CGT CTC GTC CTT GGC A 3' S' TCA AAT ACA CCA ATG TGC C 3' 5' CTA CGT GCA CCA CCA TTT TG 3' 5' CAA ACG GAA CAT GGT CCC 3' S' TCG TTC TCC CAA GGC TTG 3' 5' ATG AAA CGC GAC CTC CC 3' 5' TTC TCC ACT AGC CCC GC 3' 5' TCT CCC GGA GGG TAG GAG 3' 5' ATG TGC ATG TCG GAC GC 3' 5' AAA CTT AGA ACT GTA ATT TCA GA 3' S' GAT CAA GAC TIT TGT ATC TCT C 3' 5' ATC AAC AAG GTT TGT GTG TTG G 3' S' CCT ATG GTC TCC ATC ATG AGG 3' S' TTT GTT GGG GGT TAG GAT TAG 3' S' AAG TTT CAC ACA AGA TCT CTC C 3' 5' ATA GCG AAG TCT CCC TAC TCC A 3' S' TTG TAC ATT AAG TTC CCA TTA 3' 5' TGC TTG TCT AGA TTG CTT GGG 3' S' ATG AGT TCC GCC AAA GAA TG 3' 5' GGG TCT TCA TCC GGA ACT CT 3' 5' CCC ATA CGA TGA TGT GTT TCC 3' S' CTA CAA TTC GAA GGA GAG GGG 3' S'TCA TCT GCT ATT TGT GCT ACA 3' S' TGT CAT GGA TTA TTT GGT CGG 3' S' ATC ATG TCG ATC TCC TTG ACG 3' 5' TAC AAC CGC AAG TAA TGC CA 3' 5' TCG ATT TAT TTG GGC CAC TG 3' 5' GGG CTA GAA AAC AGG AAG GC 3' 5' GTC AGA TAA CGC CGT CCA AT 3' 5' ACG CCA GTT GAT CCG TAA AC 3' 5' TTT TCA TTG TGC CCT CTA CT 3' S' GCT TGA GAC CGG CAC AGT 3' S' CGA GGC AGC GAG GAT TT 3' S' GAG CCC ACA AGC TGG CA 3' 5' GAT CTC CCA TGT CCG CC 3' 5' GTG CTG CCA CCA CTT GC 3' S' CGA CAT TGG CTT CGG TG 3' **WMS445** WMS434 VMS440 WMS443 WMS428 WMS429 WMS437 WMS415 WMS403 WMS411 WMS413 WMS425 WMS427 WMS395 WMS410 WMS412 WMS408 WMS393 WMS400 WMS390 WMS397 WMS376 WMS388 **WMS384** WMS389 WMS391 WMS382 VMS383

GA GA CA GA CT CA GTimp GA CT CA GT CA GT CA GT CA GT CA GT CA GT CA GT CA CT CA GT CA CT CA CT CA CT CT CA CT CT CT CT CT CT
5' CAC ATG GCA TCA CAT TTG TG 3' 5' ACG GAG AGC AAC CTG CC 3' 5' TGC TCT CTC TGA ACC TGA AGC 3' 5' TGC TCT CTC TGA ACC TGA AGC 3' 5' CGA TAA CCA CTT CTC G3' 5' GCT TGC CAG TTC CAC ACC 3' 5' CAC CCC CTT GTT GGT CAC 3' 5' CAC CCC CTT GTT GGT CAC 3' 5' TTG CTG GTA GCT TCA ATC CC 3' 5' TTC CTG GTA GCT TCA ATC CC 3' 5' TTC CTG GTA GCT TCA ATC CC 3' 5' TCC TG GTA GCT TCA ATC CC 3' 5' TCC TG GTA GCT TCA ATC CC 3' 5' TCC AAA GTT GGG TGA TAT AC 3' 5' TCC AAA GTT GGG TGA TAT AC 3' 5' GGG GAG TGG AAA CTG CAT AA 3' 5' GGG GAG TGG AAA CTG CAT AA 3' 5' GGG GAG TGG AAA CTG CAT AA 3' 5' GGG GAG TGG CAG TTT GGC C' 3' 5' GGG GAG TGG CAG TTT GGC C' 3' 5' GGT CTG TTC ATG CCA CAT TG 3' 5' CAG GGT GGT GCA TGC CTC A 3' 5' CAG GGT GGT GCA TGC CTC A 3' 5' TCA ACT TCT TGG CCT CCA TC 3' 5' TCA ACT TTT GTG TCG TTC CT 3' 5' GGC ACT TTT GTG TCG TTC CT 3' 5' GGC ACT TTT GTG TCG TTC CT 3' 5' AGG CAT TGT ATA CGT TAA GGG G3' 5' AGG CAT TTT GTG TCG TTC CT 3' 5' AGG CAT TTT GTG TCG TTC CT 3' 5' AGG CAT TTT GTG TCG TTC CA AAT T3' 5' AGG CAT GGA TAG AGG GGC 3' 5' AGG CAT GTA ATA CGT TAA AGG G3' 5' AGG CAT TTT GTG TCG TTC CAA AAT T3' 5' AGG CAT TGT GTG TAC AAG CCT CAA AAT T3' 5' CAT TGT TGT GTG TAC AAG CCT CAA AAT T3' 5' CAT TGT TTGT GTG CAAG GGC 3'
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- 6. A method for the preparation of a microsatellite marker of claims 1 to 5 for plants of the Triticum aestivum species as well of the Tribe Triticeae, characterized in that hypervariable genome sections (so-called microsatellites), with the help of the polymerase chain reaction (PCR), are amplified, subsequently separated and detected to polymorphous fragments in the presence of two specific primers, which flank a microsatellite sequence to the left and right of each microsatellite locus.
- 7. The method of claim 6, characterized in that highly resolving agarose gels, native polyacrylamide gels or denaturing polyacrylamide gels are used for the separation of the markers.
- 8. The method of claim 6, characterized in that, depending on the separation system, the detection is carried out by means of ethidium bromide staining, silver staining, radiographic labeling followed by autoradiography or by means of automatic sequencing equipment using dye- or fluorescence-labeled primers.
- 9. The use of the microsatellite markers of claims 1 to 7, for the genetic analysis of hexaploid and tetraploid cultivated forms of wheat.
- 10. The use according to claim 8 for the genetic mapping and marking of monogenic and polygenic properties and their selection for analyzing relationships and identifying varieties, as well as for evaluating the purity of varieties, identifying hybrids and breeding plants.

NAME OF PERSON SIGNING: TITLE OF PERSON OTHER THAN OWNER: Acting Director Admin Director Appress OF PERSON SIGNING: Total the page 100 Person Signing: Total	Applicant or Pa Serial or Paten Flied or Issued For:	t Number:	Attorney's Docket No.
I hersby declare that I am I the owner of the small business concern identified below an official of the small business concern identified below an official of the small business concern empowered to act on behalf of the concern identified below NAME OF CONCERNInstitut für Pflanzengenetik und Kulturpflanzenforschung		Verified Stateme	nt (Declaration) Claiming SMALL ENTITY
the owner of the small business concern identified below an official of the small business concern empowered to act on behalf of the concern identified below NAME OF CONCERNInstitut für Pflenzengenetik und Kulturpflanzenforschung		Status (37 CFR 1.9	(f) and 1.27 (d)) - Small Business Concern
NAME OF CONCERNinstitut für Pfenzengenetik und Kulturpflanzenforschung ADDRESS OF CONCERN _Correnestrases S, D-06466 Gateraleben, Germany I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-16, and reproduced in 37 CFR 1.3(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its stillates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous facel year of the concern of the persons employee of at hull-filme, part-time or temporary basis during each of the pay periods of the facel year, and (2) concerns are stillates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both. I hareby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled _Microsatellita Markers for Pfants of the Species Tritioum Aestivum and Tribe Tritioes and the Use of Sald Markers by inventor(s) _Marion Röder, _Jens Plaschke; and Martin Ganaldescribed in If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is lated below* and no rights to the invention are held by any person, other than the invention is lated below* and no rights to the invention are held by any person, other than the invention is lated below* and no rights to the invention are held by any person, other than the invention are required from each named person, concern or organization having rights to the invention averring to their status as a small entities.	hereby declare	that I am	
i hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and (p) of Title 95, United States Code, in that the number of employees of the cencern, including those of its stiffliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both. I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitledMicrosatelitis Markers for Plants of the Species Tritioum Aesthum and Tribe Tritioase and the Use of Said Markers for Plants of the Species Tritioum Aesthum and Tribe Tritioase and the Use of Said Markers for plants of the Species Tritioum Aesthum and Tribe Tritioase and the Use of Said Markers for plants of the Species Tritioum Aesthum and Tribe Tritioase and the Use of Said Markers for June 19 patent no	an official of	of the small business (of the small business o	concern identified below concern empowered to act on behalf of the concern identified
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if the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27) NAME: ADDRESS: [] Individual [] Small Business Concern [] Nonprofit Organization I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)) I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 15 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed. NAME OF PERSON SIGNING: Prof. Dr. Ulrich Wobus Bernd Ei TITLE OF PERSON SIGNING: Prof. Dr. Ulrich Concertion and Crop	business conce	rn identified above wit recies Triticum Asstivu	th regard to the invention, entitledmicrosatelise markers for im and Tribe Triticeae and the Use of Said Markers
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ADDRESS: [] Individual [] Small Business Concern [] Nonprofit Organization NAME: ADDRESS: [] Individual [] Small Business Concern [] Nonprofit Organization I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)) I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed. NAME OF PERSON SIGNING: Prof. Dr. Ulrich Wobus Bernd Einter of PERSON SIGNING: The Constinct and Constitution and Constin	*NOTE	concern or of	rganization having rights to the invention averting to their
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Wall Street Tower

20 Exchange Place - 37th Floor
New York, New York 10005
United States of America

If each inventor understands English, the Declaration and Power of The Attorney below is suitable for use when filing regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

CAVEAT:

Please read accompanying INFORMATION SHEET before signing

COMBINED DECLAR APPLICATION	2930.10400					
believe am the or	office address and c ginal, first and sole plural names are lis	itizenship are as stated Inventor (if only one na ited below at 201-205) :	l below next to my nami me is listed below at 20 of the subject matter wh	11) or an original firet		
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the specification of v	vhich (check one)					
is attached he	areto					
X was filed on27 June 1996						
under Serial	NumberPCT/DE	96/01185				
and was ame	ended on	(if applic	sbie).	·		
I hereby state that I ! the claims, as amend	have reviewed and a ded by any amendr	understand the content nent referred to above.	of the above Identified	specification, including		
		mation which is material al Regulations, Section	il to the examination of	this application in		
I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:						
Application Number		Country	Filing Date (day,month,year)	Priority Claimed under 35 USC 119		
195 25 284.5						
YES: NO:						
I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.						
Application No. Filing Date						
I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:						
Bruce S. Londa (33,531). Brian L. Wamsley (33,045) Alex L. Yip (34,759)						
Alex L. Yip (34,759) Family Name RODER City of Residence State or Foreign Country of Citizenship						
201	City of Residence		State or Foreign Country Germany	Country of Citizenship Germany		
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	Post Office Address City State & ZIP/Country					
Reuthestrasse 9 D-06507 Rieder Germany						

202	Family Name PLASCHKE	First Given Name Jens	Second Given Name
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	Post Office Address	City	State & Zip/Country
	AMücke-Ring 12B	D-01662 Meissen	Germany
203 ³ -0	Family Name GANAL	First Given Name Martin	Second Given Name
203		State or Foreign Country	Country of Citizenship
	Rieder	Germany DEX	Germany
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	Family Name	First Given Name	Second Given Name
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	Family Name	First Given Name	Second Given Name
205	City of Residence	State or Foreign Country	Country of Citizenship
	Post Office Address	City	State & ZIP/ Country
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Signature of inve		Date Jan. 7	Kr. 1998 12 1998
Signature of Inve	CA COV	Date Jan 1	12 1998

Signature of inventor 201	Marion Michel	Date	Jan. 7ks, 1998
Signature of Inventor 202	Lens Clarke	Date	Jan. 12 1998
Signature of Inventor 203	Mutin Ganal	Date	Jan. 7th, 1998
Signature of inventor 204	J	Date	
Signature of Inventor 205		Date	

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PATENTS

MAILING CERTIFICATION

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 2023l on April 29, 1998

Bruce S. Londa

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No. 2936.104/00

Applicant

: Marion Roder

Appln. Number

: 08/983,605

Filed

: 12/29/97

For:

Microsatellite Markers for Plants of the Species Tritcum Aestivum and Tribe

Triticeae and the Use of Said Markers

BOX PCT

Hon. Assistant Commissioner of Patents

Washington, D.C. 20231

Sir:

Applicant submits herewith the Declaration required under 37 CFR 1.63.

Kindly charge the surcharge of \$65.00, applicant(s) being entitled to Small Entity Status on the basis of Verified Statement(s) filed February 13, 1998, to Account No. 04-2216.

The Commissioner is hereby authorized to charge any additional fees which may be required to make this response timely, or credit any overpayment to Deposit Account 04-2216.

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